

# **Molecular Studies of the Role of Protectants in Cryopreservation and Lyophilization of Biological Systems**

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In recent years, considerable efforts have been directed towards the cryopreservation and lyophilization (or freeze-drying) of biological systems. The overall goal of those efforts has been to develop effective processes and formulations for long-term storage of proteins, cells, and other systems of pharmaceutical or biomedical interest. Most cryopreservation and lyophilization media require use of cryo- or lyoprotectants; these are molecules which are believed to stabilize the native structure of proteins in solution, to inhibit the formation of ice crystals, and to preserve the structural integrity of cell membranes in anhydrous samples. Disaccharides such as sucrose and trehalose have been shown to be particularly effective in this regard. However, little is known about the precise molecular-level mechanisms responsible for their effectiveness. In this work we present a detailed molecular simulation study of the structure and thermophysical properties of cryoprotectants in biologically relevant systems. Three types of systems are considered: aqueous solutions over a wide concentration range, protein solutions, and hydrated and anhydrous lipid bilayers. We examine the proposed depletion of these molecules from the vicinity of proteins, and we propose structural arguments which help explain the origin of the so-called 'preferential exclusion principle.' We consider the interaction of disaccharides with lipid bilayers, and a mechanistic model is proposed to explain the role played by these molecules in the stabilization of anhydrous cell-lyoprotectant matrices. We also apply advanced computational methods (Hybrid Density of States) to determine the potential of mean force of cryoprotectants through the lipid bilayers, and suggest the mechanism responsible for the stabilization of bilayers.